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Sperm investment in male meadow voles is affected by the condition of the nearby male conspecifics

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Abstract.

Sperm competition occurs when two or more males copulate with a particular female during the same reproductive cycle, and their sperm compete to fertilize the female's available eggs. One strategy that male voles use to assess the risk and intensity of sperm competition involves responding to the presence of scent marks of conspecific males found near a sexually receptive female. Previously, we have shown that if a male vole copulated with a female while he was in the presence of the odors of another male he increased his sperm investment relative to his investment if another male's odors were not present. The aim of the present study was to test the hypothesis that males assess differences in the relative quality of competing males and adjust their sperm investment accordingly. We did so by allowing males to copulate when they were exposed to the scent mark of a 24-h food-deprived male (low-quality male) or the scent mark of a male that was not food deprived (high-quality male). The data indicate that male meadow voles did not increase their sperm investment during copulation when exposed to the scent marks of a food-deprived male, but did so when they were exposed to the scent marks of males that were not food deprived. The results support the hypothesis that male voles are able to adjust sperm investment when they encounter the scent marks of males that differ in quality.

Key Words**:** copulatory behavior, food deprivation, voles, scent marking, chemical signals, sperm competition

Sperm competition occurs when two or more males copulate with a particular female during the same reproductive cycle, and their sperm compete to fertilize the female's available eggs (Smith 1984; Birkhead and Møller 1998; Birkhead 2000; Simmons 2001). There are more than 95% of mammalian species that show some degree of promiscuity (Kleiman 1977), and sperm competition has been found to be prevalent in mammals (Ginsberg and Huck 1989; Gomendio et al. 1998). The frequent occurrence of sperm competition may have forced males to develop different strategies to reduce the risk of displacement of their own sperm by competing males, and to displace or overcome the sperm of competing males (Huck et al. 1985). One strategy for overcoming the sperm of other males is by adjusting the amount of sperm allocated to the ejaculate (Parker et al. 1996; Williams et al. 2005). Males may increase their sperm investment in response to the risk of sperm competition (Parker et al. 1996) as shown by the bush cricket, *Kawanaphila nartee* (Simmons and Kvarnemo 1997), the house cricket and the decorated cricket, *Acheta domesticus* and *Gryllodes supplicans* (Gage and Barnard 1996), the white butterfly, *Pieris rapae* (Wedell and Cook 1999), the bitterling, *Rhodeus sericeus* (Candolin and Reynolds 2002; Smith et al. 2003), the black goby and sneaker males of the grass goby, *Gobius niger* and *Zosterisessor ophiocephalus* (Pilastro et al. 2002), territorial gobies (Scaggiante et al. 2005), parental bluegill sunfish, *Lepomis macrochirus* (Neff et al. 2003), Norway rats, *Rattus norvegicus* (Pound and Gage 2004), and meadow voles, *Microtus pennsylvanicus* (delBarco-Trillo and Ferkin 2004, 2006a). Alternatively, males may not adjust sperm investment as the risk of sperm competition increases as described in a species of cricket, *Gryllus texensis* (Schaus and Sakaluk 2001)

and the quacking frog, *Crinia georgiana* (Byrne 2004). Finally, male house mice, *Mus musculus domesticus* may reduce their sperm investment if the risk of sperm competition increases (Ramm and Stockley 2007).

During the breeding season, male meadow voles occupy large home ranges that encompass the territories of one or more females. Females inhabit mutually exclusive territories (Madison 1980). Male and female meadow voles are promiscuous and most interactions between opposite-sex conspecifics are limited to mating attempts (Madison 1980; Boonstra et al. 1993). Despite the high frequency of encounters between males and females, encounters between same-sex conspecifics, particularly between males, are less frequent (Madison 1980). Male-male agonism is not common (Ferkin and Seamon 1987) and when it occurs males do not establish dominance hierarchies (Ferkin 2007). Thus, male voles do not directly restrict other males from having access to sexually receptive female voles, and therefore the incidence of sperm competition is likely to be high (Dewsbury 1981; Boonstra et al. 1993; Berteaux et al. 1999). Consequently, male voles are likely to have developed physiological, morphological and/or behavioral strategies to confront the normal occurrence of sperm competition (Dewsbury 1981; Boonstra et al. 1993).

One strategy that male voles use to allocate sperm during copulation is to assess the risk and intensity of sperm competition by the presence of scent marks of conspecific males found near a sexually receptive female, which may be a good estimate of the number of males that will copulate with that female (Salo and Dewsbury 1995). Our recent work has supported and expanded this hypothesis by showing that if a male

meadow vole is paired with a female vole and both are exposed to the odor of a male conspecific, the copulating male will increase his sperm investment by over 116% (delBarco-Trillo and Ferkin 2004). A male vole's sperm investment, however, does not rise as high if he is exposed to the scent marks of several males (delBarco-Trillo and Ferkin 2006a), suggesting that male voles are able to assess differences in the number of potential mates near a receptive female. Interestingly, the male did not alter his sexual behavior (delBarco-Trillo and Ferkin 2004, 2006a-c, 2007) as has been shown in other animals (Stockley and Preston 2004). Given that male meadow voles adjust their sperm investment during mating when exposed to the scent marks of other males, it begs the question as to whether they adjust their sperm investment based on the information contained in the scent marks of competing males. For example, do males adjust their sperm investment if they encounter the scent marks of males that differ in some feature of their quality?

The aim of the present experiment was to determine whether males assess differences in the relative quality of competing males and adjust their sperm investment accordingly. We selected males that were not food deprived and males that were food deprived as odor donors to represent differences in their relative quality and resultant risk of sperm competition. Recent work has reported that food-deprived male voles may be of "lower quality" relative to males that were not food deprived (Pierce and Ferkin 2005). First, food-deprived males produced odors that were less attractive to sexually receptive females than those of males that were not food deprived. Next, food-deprived males spent less time than males that were not food deprived investigating the odors of

receptive females. Lastly, food-deprived males engaged in coitus fewer times than males that were not food deprived when paired with a sexually receptive female conspecific (Pierce and Ferkin 2005; Pierce et al. 2005). Thus, males that are food deprived may produce odors or scent marks that are associated with a decreased risk of sperm competition, whereas odors or scent marks from males that were not food deprived may represent a risk of sperm competition. If so, a prediction of the hypothesis is that a copulating male will increase his sperm investment if he encounters the scent mark of a male conspecific that was not food deprived for 24 h, but will not increase his sperm investment if he encounters the scent mark of a male that was food deprived for 24 h. Such a finding would suggest that males are able to adjust their sperm investment when they encounter males that represent different risks of sperm competition.

Methods

Animals

The meadow voles used in this study were offspring of field-caught animals, all of which were born and raised at The University of Memphis in a room that was controlled for temperature and on a 14:10 hour light-dark cycle to simulate day length during breeding season. Meadow voles are weaned at 19 days of age and kept with littermates until they are 34 days old. They are then housed singly in clear polycarbonate cages (27 x 16.5 x 12.5 cm). Cages contain hardwood shaving as bedding and cotton for nesting material. Food and water are provided *ad libitum* (except for odor donors in the food-deprived condition, as explained below).

Treatment Groups

Thirty-six male and 36 female meadow voles were used in this study, with 12 different males and 12 different females used in each sperm competition treatment group. This resulted in 36 pairs of voles being used in the experiment. Adult male meadow voles copulated with sexually receptive females in one of three groups that only differed in the type of scent mark the copulating male was exposed to during the trial. In one group (n = 12 male-female pairs), we paired a female and a male vole who mated in the presence of no scent marks from a conspecific male; this group represented the control condition (CONTROL). In the control condition water was used instead of a scent mark. In the second group (n = 12 male-female pairs), we paired a male and female in the presence of the scent mark of a male that was food deprived for 24 h (FD-M). As mentioned earlier, this group represents the scent marks of males considered to be of lower quality relative to the copulating male. In the third group (n = 12 male-female pairs), we paired a female and male vole in the presence of the scent mark of a male that was not food deprived for 24 h; this male scent donor had continuous access to food (1M). This group is similar to that described in delBarco-Trillo and Ferkin (2004, 2006a) in that it represents the scent marks of males considered to be of similar quality to the copulating male.

Testing Procedure

We used control (fresh water) and fresh male scent marks for each male-female pairing using methods detailed elsewhere (Ferkin et al. 1999; Pierce et al. 2005). Briefly,

in the control condition fresh distilled water was placed on a sterile cotton applicator and rubbed for five seconds on the center portion of a clean glass microscope slide (7.5 cm x 2.5 cm). In the food-deprived (FD-M) and non-food-deprived conditions (1M), the anogenital area of the male scent donor was rubbed against the center portion of a clean glass slide for five seconds. The resulting scent marks from the male donors and the water mark were roughly the same size, approximately 1.2 cm x 0.3 cm (l x w). We used a single slide for each pairing. A different male's scent mark was used in each trial and each donor was only used once (n = 12 FD-M donors and n = 12 1M donors). None of the male scent donors were familiar or related to the copulating male. However, all male scent donors and copulating males were similar in age (between 6-9 mo old), weight (within 8 g), and sexual experience (having previously sired a litter).

Immediately after the scent mark slide was prepared, we placed a female vole into the testing cage (37 x 21 x 15 cm). The female voles were injected with 0.05 mg of estradiol 60 h prior to pairing to increase the chance that the females would be receptive and mate (delBarco-Trillo and Ferkin 2004). Five minutes after the female was placed in the cage, we placed a glass slide containing a scent mark of a male donor or the control into the cage. The slide was suspended 2 cm above the substrate by a clean metal clip and hook. Five minutes after the slide was placed into the cage, we placed the subject male into the cage. We allowed these males to mate until sexual satiety, which is 30 min without any intromission (Gray and Dewsbury 1975; delBarco-Trillo and Ferkin 2004).

We recorded copulatory behavior of voles using methods similar to those detailed elsewhere (delBarco-Trillo and Ferkin 2004). Briefly, copulatory behavior of voles was

recorded using a video-camcorder connected to a VCR recorder. We later scored the tapes to determine the total number of ejaculations, the latency to first ejaculation, and the mean ejaculation interval. The latency to first ejaculation was the amount of time (seconds) from the start of the trial to the first ejaculation. The mean ejaculation interval was the average amount of time (seconds) between each ejaculation. The methods for scoring these two variables are similar, but not exactly the same as was seen in an earlier paper examining copulatory behavior in meadow voles (delBarco-Trillo and Ferkin 2007). The scorers of the videotapes were blind to the treatment group of the voles.

Immediately after the male reached sexual satiety, he was removed from the cage and returned to his home cage, the glass slide was discarded, and the female was removed from the cage and euthanized using an overdose of Isoflurane vapors. The female reproductive tract was removed, opened and all the semen diluted in 25 ml of distilled water as detailed in delBarco-Trillo and Ferkin (2004, 2006a). The solution was gently homogenized. Four sperm counts were conducted using an improved Neubauer hemocytometer. The average of the four sperm counts was used to estimate the total number of sperm ejaculated by the male or his sperm investment (delBarco-Trillo and Ferkin 2004, 2006a). The sperm counter was blind to the treatment group being tested.

Statistical analyses

The experimental design of this study is more similar to that of delBarco-Trillo and Ferkin (2006a) than it is to the earlier delBarco-Trillo and Ferkin study (2004) in that we do not use a "within-animal" design in the current study. This was due to difficulty of

obtaining three successful trials with the same male. Generally, not using a within-animal design may be a problem in this type of study if there is much unexplained variation among males (Pound and Gage 2004). However, previous work has shown that much of the variation in sperm investment of male voles is explained by male body size (delBarco-Trillo and Ferkin 2004) and therefore may be controlled by incorporating male body size in the statistical analyses as a covariate.

It has been previously reported that sperm investment is significantly correlated with male body weight (delBarco-Trillo and Ferkin 2004). Therefore, we used an ANCOVA to control for the effect of male body weight on sperm investment (delBarco-Trillo and Ferkin 2006a). The grouping variable was treatment group (CONTROL, 1M, and FD-M), and the covariate was male body weight. Before running the ANCOVA, we tested whether the assumption of homogeneity of regression was met using a Kolmogorov-Smirnov test. Levene's homogeneity of variance test was used to test the assumption of homoscedasticity. We used ANCOVA, the covariate being male body weight, with a Fisher's least significant difference adjustment for the pairwise comparison (delBarco-Trillo and Ferkin 2006a). Statistical analyses were performed using SPSS 16 for Windows. Differences were considered significant at $p < 0.05$. We also used one-way analysis of variance (ANOVAs) to determine whether males in the different treatment groups had different numbers of ejaculations, latencies to first ejaculation, and mean ejaculation intervals. The independent variable was treatment group (CONTROL, 1M, and FD-M). The dependent variable was the number of ejaculations, latency to first ejaculation, or the mean ejaculation interval.

Results

We found significant differences in sperm investment between the three groups (ANCOVA: $F_{2,32} = 6.213$, $p = 0.005$; Fig.1). Sperm investment was lowest in the CONTROL group, which was statistically similar to the FD-M group ($F_{1,32} = 0.028$, $p = 0.868$). The highest sperm investment was in the 1M group (Fig. 1). A significant difference was found between the CONTROL and 1M groups, with the 1M males having a significantly higher sperm investment ($F_{1,32} = 9.79$, $p = 0.005$). There was also a significant difference between the FD-M and 1M group, with the 1M males again investing more sperm ($F_{1,32} = 5.827$, $p = 0.025$). Although we controlled for body size of males, a subsequent analysis revealed that it did not affect sperm investment in male voles. The ANOVA results also showed a difference between the three groups $F_{2,33} = 5.984$, $p = 0.006$. The Tukey post-hocs also showed a similar result, there was a significant difference between the CONTROL and the 1M group and also between the 1M group and the FD-M group (both comparisons, $p < 0.05$).

We found that different risks of sperm competition did not affect aspects of the copulatory behavior of male voles. There was not a significant difference among the three different treatment groups in the number of ejaculations (6.03 ± 0.36 ejaculations; $F_{2,33} = 0.771$, $p = 0.471$; Fig. 2a), latency to first ejaculation (1704.7 ± 453.1 s; $F_{2,33} = 1.095$, $p = 0.347$; Fig. 2b), and mean ejaculation interval (979.6 ± 100.9 s; $F_{2,33} = 0.238$, $p = 0.790$; Fig. 2c). Typically, male and female voles completed their mating bouts within 40 min-3.5 h of being paired.

Discussion

Differences in male quality were established by selecting male voles that were not food deprived or that were food deprived for 24 h prior to testing. Previous work has shown that food-deprived male voles may be of "lower quality" relative to males that were not food deprived. Briefly, male voles that were food deprived for 24 h produced odors that were less attractive to females, spent less time investigating the odors of receptive females, and were less likely to copulate than males that were not food deprived (Pierce et al. 2005). Our results show that males are able to adjust their sperm investment when they encounter the scent marks of males that were not food deprived for 24 h but do not increase their sperm investment during copulation when they are exposed to the scent mark of a male that was food deprived for 24 h. Indeed, sperm investment was similar in the presence of the scent mark of a food-deprived male and in the absence of any scent marks from male conspecifics. These findings suggest that food-deprived males may represent a reduced risk of sperm competition relative to males that were not food deprived. Our results are consistent with those of previous studies showing that sperm investment of a copulating male mammal will increase if he encounters the scent marks of a conspecific male of similar relative quality, which represents a stronger risk of sperm competition (delBarco-Trillo and Ferkin 2004, 2006a; Pound and Gage 2004). Males also increase their sperm investment when the risk of sperm competition is high as seen in the white butterfly (Wedell and Cook 1999), the house cricket and the decorated cricket (Gage and Barnard 1996), and the black goby and sneaker males of the grass goby

(Pilastro et al. 2002). More importantly, our study extends the hypothesis that male mammals can assess the risk and intensity of sperm competition (delBarco-Trillo and Ferkin 2004, 2006a; Pound and Gage 2004) by showing that male mammals can assess the relative quality of nearby males and use the information found in their scent marks to adjust their own sperm investment.

Our present findings and those from previous studies demonstrate that male voles can allocate different amounts of sperm when they encounter males that represent different relative risks of sperm competition (this study; delBarco-Trillo and Ferkin 2004, 2006a). The ability to adjust sperm investment depending on both the relative risk of sperm competition and the intensity of sperm competition may be a strategy employed by males to use sperm prudently (Parker 1970; Dewsbury 1982; Dewsbury and Sawrey 1984; Parker et al. 1996). If there are multiple competitors, then the likelihood of siring the offspring of a particular female will decrease. The ability to adjust sperm investment may be an advantage to individuals in species characterized by a promiscuous mating system (Birkhead 2000), a social system where male mammals visit the territories of females that likely contain the scent marks of males that are able to represent different relative risks of sperm competition (Madison 1980; Boonstra et al. 1993; Ferkin and Pierce 2007), a high incidence of sperm competition (Dewsbury and Sawrey 1984; Gomendio et al. 1998; Berteaux et al. 1999), and an environment containing variable food availability (Getz et al. 2001). It is worth mentioning that multiple mating may occur in other species of voles, including those species that have mating systems characterized by either polygyny or monogamy (Wolff and Dunlap 2002; Klemme et al.

2006). It would be interesting to know if males in these species make similar sperm allocation adjustments when they encounter the scent marks of conspecific males.

Male meadow voles did not adjust aspects of their copulatory behavior when they were exposed to males that represent different risks of sperm competition. This result is interesting because males in many other species do adjust copulatory behaviors according to risk of sperm competition. Much evidence suggests that when faced with a high risk of sperm competition males alter their copulatory behavior in such a way as to increase the likelihood that they will fertilize the female's eggs (Stockley and Preston 2004). In rats it has been found that increasing the intromission length leads to more vaginal stimulation of the female (Adler and Toner 1986). It may also cause a reduction in female receptivity, which may reduce the future risk of a male competitor mating with that particular female (Hardy and DeBold 1972; Stockley and Preston 2004). Roof rats, *Rattus rattus,* and montane voles, *Microtus montanus,* have been found to decrease the latency to copulate when there is a perceived risk of sperm competition (Shapiro and Dewsbury 1986; Estep 1988). In contrast, our results showed that for male meadow voles the number of ejaculations, the latency to first ejaculation, and the mean ejaculation interval did not differ significantly across treatment conditions. Similar results have also been reported in other experiments on meadow voles, showing that males exposed to different risks and intensities of sperm competition do not alter their copulatory behavior (delBarco-Trillo and Ferkin 2004, 2006a, 2007). For male meadow voles, it appears that the number of ejaculations and other aspects of copulatory behavior in a mating bout may be somewhat fixed. The lack of change in the copulatory behavior of male voles in the

face of different risks of sperm competition may provide males with benefits that outweigh the costs. Male and female meadow voles are promiscuous and can mate with multiple partners during a breeding event (Boonstra et al. 1993; Berteaux et al. 1999). To increase the likelihood of reproductive success, males must provide females, which are induced ovulators (Milligan 1982), with sufficient vaginal stimulation during coitus to ensure she ovulates and he must provide sufficient sperm to increase his chances of getting the female pregnant (Gray and Dewsbury 1975; Seabloom 1985; Bakker and Baum 2000). If there are too few intromissions and ejaculations, the female may not ovulate and become pregnant. If the number of intromissions and subsequent ejaculations are sufficient to allow a female to become pregnant, males may not need to increase the number of ejaculations they have with a particular female, especially if by doing so, he reduces the likelihood that he can impregnate additional females. As seems to be the case for meadow voles, a better strategy than modifying the number of ejaculations that males have during a copulatory bout with a female may be to adjust the number of sperm per ejaculation. This adjustment of sperm investment, especially during the first ejaculations, may account for the uncertainty of whether a male meadow vole will be able to complete a full mating bout with a given female (delBarco-Trillo and Ferkin 2006a, c, 2007).

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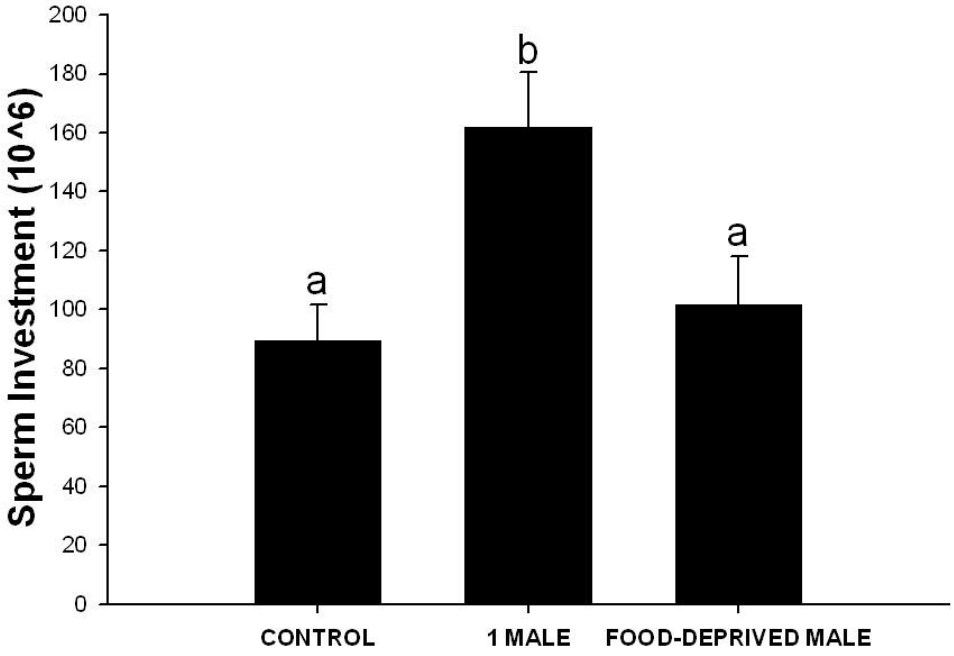

Figure 1. The mean + SEM sperm investment of copulating males exposed to a clean glass slide (control), a glass slide containing the scent mark of an unrelated, unfamiliar male conspecific (1M), and a glass slide containing the scent mark of an unrelated, unfamiliar male conspecific that was food deprived for 24 h (FD-M). Histograms capped with different letters are significantly different at $p < 0.05$.

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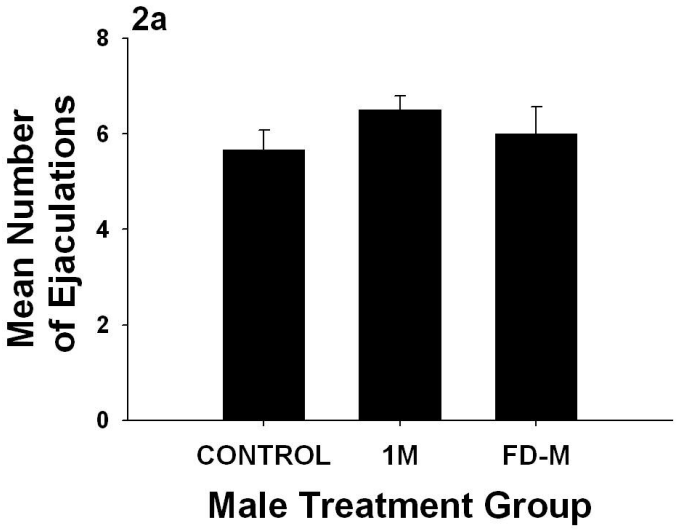

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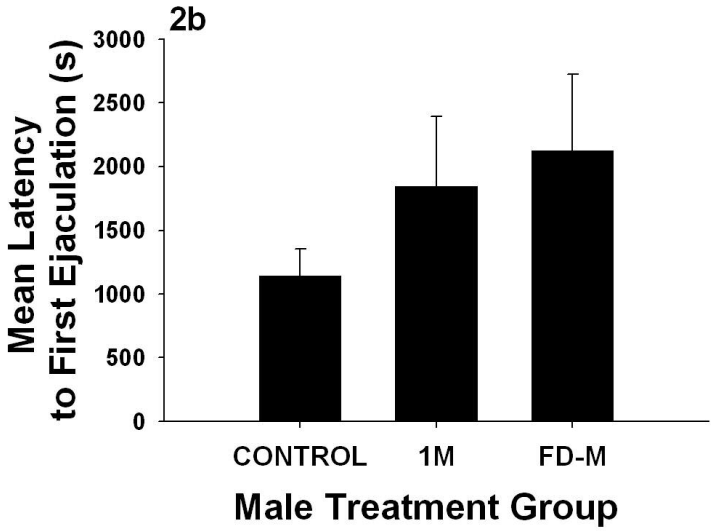

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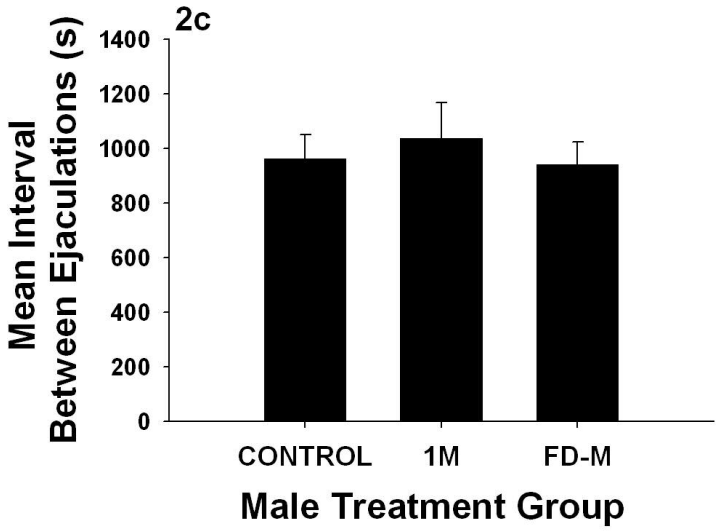

Figure 2. The mean + SEM number of (a) ejaculations by males, (b) latency (seconds) to first ejaculation, and (c) mean interval (seconds) between ejaculations by males exposed to a clean glass slide (control), a glass slide containing the scent mark of an unrelated, unfamiliar male conspecific (1M), and a glass slide containing the scent mark of an unrelated, unfamiliar male conspecific that was food deprived for 24 h (FD-M). There were no significant differences between the groups of males.
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Sperm investment in male meadow voles is affected by the condition of the nearby male conspecifics.

Ashlee A. Vaughn; Javier delBarco-Trillo; Michael H. Ferkin

Male mammals may use different tactics to increase the likelihood that their sperm fertilizes a female's eggs. Male meadow voles increase the amount of sperm in the ejaculate when they encounter the scent marks of other male voles near a receptive female. If they encounter no scent marks of other males, they do not increase the amount of sperm in their ejaculate. The aim of the present study was to test the hypothesis that males assess differences in the quality of males that deposit scent marks near receptive females and alter their sperm investment accordingly. That is, increase sperm investment if the other male is viewed as being of high quality and not to do so if the other male is viewed as being of low quality. We tested the hypothesis by measuring the amount of sperm in the ejaculate of males that mated with a female that was next to the scent marks of a male that was food deprived for 24 h (low quality male), next to the scent marks of a male that was not food deprived (high-quality male), or next to water marks. Male voles did not increase their sperm investment during copulation when exposed to the scent marks of a food-deprived male or water marks, but did so when they were exposed to the scent marks of males that were not food deprived. Male voles are able to adjust sperm investment when they encounter the scent marks of males that differ in quality.